Introduction:

Silver has been used for at least six millennia to prevent microbial infection and was important prior to the invention of antibiotics. BCE historians accounted ancient Phoenicians, Greeks, Romans, Egyptians and Persians to have used silver in one form or another to preserve food and water. Hippocrates used silver preparations for treatment of ulcers and promoted wound healings.\(^1\)

Currently, the presence of antibiotic-resistant superbugs (bugs that resist antibiotics) increases the demand for silver in hospitals. Silver in surgical equipment, wound dressings, and ointments protect wounds from infection; silver ion treatments can heal bone infections and help regenerate damaged tissues. Silver sulfadiazine is used for burn victims because it kills bacteria while also allowing the skin to regrow.

Bio toxicity of silver particles and silver based compounds on microorganisms is due to the interaction between the metals and cellular membranes. Silver binds to membrane bound proteins and destabilizes the membrane, it causes ion leakage and cell rupture. Once inside the cell, silver binds to and disrupts mitochondrial membrane and interferes with energy (ATP) yielding reactions.

Silver nanoparticles (particles that are less than 100 nm in diameter) are better than silver ions as antimicrobial agent because a large amount of silver ion would be needed which in turn maybe toxic to the microbial host. Furthermore, silver nanoparticles cause local disruption on membrane locally and the effective concentration is much less than the ionized silver.

Silver nanoparticles are created when silver ions in silver nitrate solution are reduced by the the citrate ions in sodium citrate solution. The citrate ions are oxidized and release carbon dioxide and two electrons.

**Chemical reaction:**

\[
2 \text{Ag}^+ (aq) + 2\text{-e}^- \rightarrow 2 \text{Ag(s)} \\
\text{Citrate ion} \rightarrow \text{acetone-1,3-dicarboxylate} + \text{CO}_2 (g) + 2\text{-e}^- \\
2 \text{Ag}^+(aq) + \text{Citrate ion} \rightarrow 2 \text{Ag(s)} + \text{acetone-1,3-dicarboxylate} + \text{CO}_2
\]

See Figure 1 below for the electron transfer and release of CO\(_2\).\(^2\)
Once the silver nano particles are produced, they are prevented from aggregation to bigger size. The excess citrate ions cap the silver nanoparticles as shown in the Figure 2A below. Figure 2B shows the Transmission Electron Micrograph (TEM) of the silver nanoparticles and the higher magnification image showing the crystalline planes, indicating that these are nano crystals of silver.3

Properties of materials change from nano size to bulk size. For example, nano gold particles are red and bulk gold is yellow/gold color. Nano silver particles appear to be various shade of yellow, from pale yellow to dark amber, depending the shape and size while that of bulk silver is shiny grey. The smallest silver nanoparticles are clear yellow.
Increasing in size will lead to amber and finally a cloudy brown color. The darker color solution causes less bio toxicity.

Fig. 2A – reaction between silver and citrate ion
AND capping of silver nanoparticles by excess citrate

Fig. 2B – TEM of silver nanoparticles

References:


Purpose of the lab:

The purpose of the lab is to produce silver nanoparticle solution. The solution’s biotoxicity will then be tested on yeast in the Biology 110 class. Silver nanoparticles will disrupt the respiration rate of yeast.

Materials for each group:

60.0 ml of 1mM Silver Nitrate, 6.00 ml of 10mM Sodium Citrate, 1-250 ml beaker, 100 ml graduate cylinder, 10 ml graduate cylinder, watch glass, magnetic stirring bar, 1 glass pipette, pipette bulb, thermometer, hotplate, and parafilm to cover the beaker.

Procedure:

1. Wash all glassware as dust will affect silver nanoparticle growth.

2. Label the beaker with your name, name of the solutions, molarity and the date of the preparation.

3. Measure out 60.0 mL of 1mM silver nitrate using the 100 ml graduate cylinder and put it into the 250 ml beaker.

4. Measure out 6.00 mL of 10mM sodium citrate using the 10.00 ml graduate cylinder.

5. Add a magnetic stir bar to the beaker. (*Do not use boiling stones as they’d affect nucleation.*)

6. Put the beaker on a hotplate (with built-in magnetic stirrer) and *cover it with a watch glass.*

7. Bring the solution to a boil. *If you decide to use a thermometer, clean it before you use it.* The solution will boil around 100 deg. C.

8. As soon as solution boils, add the 6.00 ml of 10mM sodium citrate dropwise, about 1 drop per second. *Do not decrease the temperature as you add the citrate solution.*
   a. Record the time when you began to add the sodium citrate
   b. When you are finished adding the sodium citrate, record the time.

Note: Once the solution starts to change color, the reaction proceeds very fast. Do not leave the solution unattended! *If you notice a silvery/black deposit on the sides or bottom of the beaker, the silver might be precipitating out, check with your instructor.*
9. Continue boiling the solution until it turns golden, as in Fig. 1 below. As long as the color of your solution is between beaker 1a (light color) and beaker 1 b (darker), there will be sufficient nanoparticles in the solution.

10. Remove from hotplate, let cool to room temperature, record the time when you removed the beaker. Once the beaker has cooled to room temperature, record the color and cover the beaker with parafilm.

Golden color of nanoparticle solution